Synthesis and characterization of Pt complexes containing dichloroacetate (DCA), designed for dual anticancer action

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In memory of Chiara Gemmo (1990-2016)

In honour of Carlo Mealli on the occasion of his 70th birthday

ABSTRACT

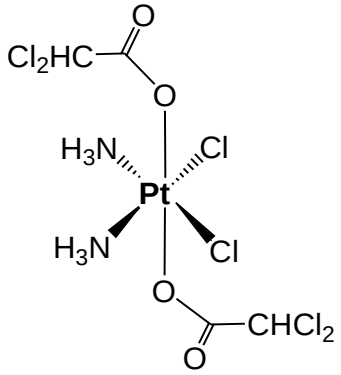
A group of new Pt(II) complexes with dichloroacetate (DCA), bearing DMSO (*cis*-[Pt(DCA)$_2$(Me$_2$SO-*S*)$_2$], **2**) or phosphines (*cis*-[Pt(DCA)$_2$(PPh$_3$)(Me$_2$SO-*S*)], **3**, *cis*-[Pt(DCA)$_2$(P)$_2$], P = PPh$_3$ **3a**, P = PTA **4a** and [Pt(DCA)(P)$_3$]DCA, P = PPh$_3$ **3b**, P = PTA, **4b**) as neutral ligands was prepared by a simple fast route from the inorganic synthon [PtCO$_3$(Me$_2$SO-*S*)$_2$], **1**. The x-ray crystal structures of **2**, **3, 3a** and **4a** were determined. The antiproliferative activity of **2**, **4a**, and **4b** was evaluated against two human cancer cell lines, cisplatin sensitive A2780 and cisplatin resistant SKOV-3, and the results were compared with known amine analogues and with the dichloride precursors.

Keywords: Pt complexes, dichloroacetic acid, x-ray crystallography, antiproliferative activity

1. Introduction

The anticancer activity of Pt complexes as well that of dichloroacetic acid are well known and different mechanisms of action have been proposed. In particular, it has been verified that Pt-drugs act as DNA alkylating agents [1] while dichloroacetate is able to trigger apoptosis in cancer cells resistant to classical anticancer drugs by selectively targeting their mitochondria [2].

The idea to include Pt and DCA in the same chemical entity with the aim of exploiting simultaneously the two actions, has been recently developed and produced the promising drug mitaplatin [3], a Pt(IV) amino-complex presently under clinical trial, which contains two residues of dichloroacetic acid. It demonstrated a stronger anticancer effect in comparison with cisplatin.



Mitaplatin

The Pt(II) analogue $[Pt(DCA)_2(NH_3)_2]$ and some Pt-DCA-1,2-DACH complexes have also been prepared and tested in vitro [4], [5].

In this work, we propose the synthesis and characterization of some non-amine Pt(II)DCA complexes, containing DMSO, PPh_3 and PTA (1,3,5-triaza-7-phosphaadamantane) as neutral ligands. Their antiproliferative activity, in vitro, has been tested on human tumoral cell lines A2780 (ovarian carcinoma, cisplatin sensitive) and SKOV-3 (cisplatin resistant). Although earlier research on Pt-drugs had considered that Pt-phosphine complexes were not active, the recent work by Messori and Weigand and by our group [6] encourages reconsideration of the pharmaceutic properties of this class of compounds.

We have recently proposed the complex $[PtCO_3(Me_2SO\text{-}S)_2]$ as a versatile synthon, where the bi-anionic chelating ligand carbonate can be replaced by carboxylates through a protonolysis process triggered by their protonated form [7]. This opened a new synthetic route to Pt-carboxylates, which present therapeutic performances improved with respect to cisplatin, in several cases [8].

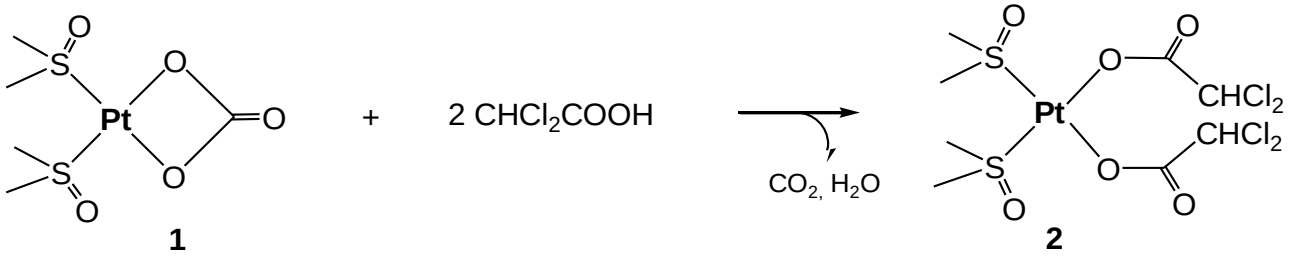
The complex $[PtCO_3(Me_2SO\text{-}S)_2]$, **1**, is easy to prepare, promptly reacts with a variety of acids releasing harmless odorless CO_2, and giving carboxylate complexes. It is then possible to replace DMSO with neutral ligands like phosphines.

In our previous paper we reported the substitution of CO_3^{2-} by bi-carboxylic or hydroxyl carboxylic acids, which produces Pt chelate complexes. With the aim of implement the versatility of complex **1** as a synthon for Pt complexes, we wished to test its reactivity with monocarboxylic acids, in order to understand whether protonolysis and consequent carboxylate coordination can occur even without formation of a chelate ring. Because of the above mentioned great interest in DCA, due to its anticancer activity [2], we choose this acid as a good candidate to be bound to Pt. In fact the expected products, Pt-DCA complexes, are likely to be provided with a dual action anticancer activity.

2. Results and discussion

*2.1. Synthesis, characterization and x-ray crystal structure of cis-[Pt(DCA)$_2$(Me_2SO-S)$_2$], (**2**)*

The reaction of [$PtCO_3(Me_2SO$-*S*$)_2$], **1,** with two equivalents of dichloroacetic acid in MeOH, gave *cis*-[$Pt(DCA)_2(Me_2SO$-*S*$)_2$], **2**, bearing *S*-coordinated DMSO as a neutral ligand. Pt-DMSO complexes have been frequently considered for their capacity to bind to nucleosides [9] and, structurally, complex **2** combines three active components (Pt, DMSO and DCA) which will hopefully result in anticancer activity through the contribute of different synergic mechanisms.
In addition, complex **2** has an interest from a synthetic point of view; in fact it can be regarded as an intermediate for the preparation of other Pt complexes via DMSO replacement or through the substitution of DCA, favored by the high *trans* effect of DMSO.



1 **2**

Complex **2** was easily obtained in high yield. The ^{1}H NMR of **2** in acetone-d_6 showed the signal of coordinated DMSO at 3.53 ppm, coupled to Pt ($^3J_{PtH}$ = 22 Hz), and that of CH of DCA at 6.15 ppm (compare with the corresponding signal of free DCA at 6.27 ppm and of its sodium salt at 6.0 ppm, in D_2O).
The ^{13}C NMR in acetone showed coordinated DMSO at 43.63 ppm and the signals of the two carbons of DCA, CH and COO, at 68.01 at 169.08 ppm respectively. A single species at -3119 ppm was observed in the ^{195}Pt NMR, in the same solvent.

The expected *cis* geometry and *S*-coordination of both DMSO ligands were confirmed by the x-ray crystal structure of **2**, determined on crystals obtained from an acetone solution (Table 1 and Fig 1).

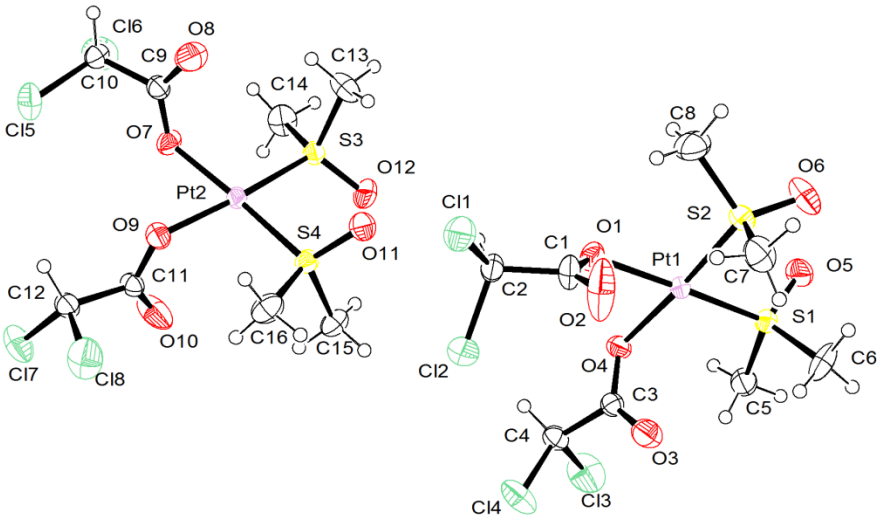
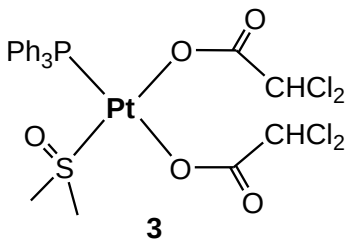


Figure 1 ORTEPIII view [10] and atom numbering scheme for *cis*-[Pt(DCA)$_2$(Me$_2$SO-*S*)$_2$], **2**. Thermal ellipsoids are drawn at the 50% probability level.

2.2. Triphenylphosphine-DCA-Pt complexes. X-ray crystal structure of cis-[Pt(DCA)$_2$(PPh$_3$)(Me$_2$SO-S)], (**3**) *and cis-[Pt(DCA)$_2$(PPh$_3$)$_2$]*, **3a.**

We found that the more convenient sequence for obtaining phosphinic-DCA complexes, by total substitution of the ligands in complex **1,** involves protonolysis with DCA giving **2** as a stable intermediate, followed by the substitution of coordinated DMSO by phosphines. These syntheses can be carried out "one pot" with no need to isolate complex **2**, thus saving time and chemicals.



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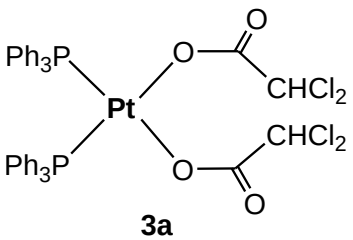
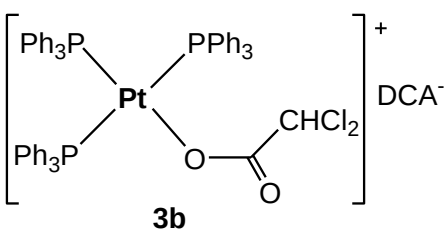


3a



3b

The new complex *cis*-[Pt(DCA)$_2$(PPh$_3$)(Me$_2$SO-*S*)], **3,** can be prepared by adding two equivalents of DCA to a solution of complex **1** in acetone (giving presumably the above described complex **2** as intermediate), followed by the addition of a single equivalent of PPh$_3$. Complex **3** was obtained and characterized by ^{31}P NMR (7.9 ppm, $^1J_{PPt}$ = 4015 Hz in acetone) and ^{1}H NMR (acetone-d$_6$), which showed two signals of coordinated DCA (at 6.18 ppm *trans* to DMSO and 5.38 *trans* to PPh$_3$) and DMSO (at 3.22 ppm, $^3J_{HP}$ = 21 Hz), beside the signals of aromatic proton of PPh$_3$ (7.50-7.90 ppm).

Crystals of complex **3**, suitable for x-ray analysis, were grown in DMSO. The crystallographic analysis confirmed the presence of an S-coordinated DMSO, one PPh$_3$ and two DCA anions in a *cis* configuration (Table 1 and Fig. 2).

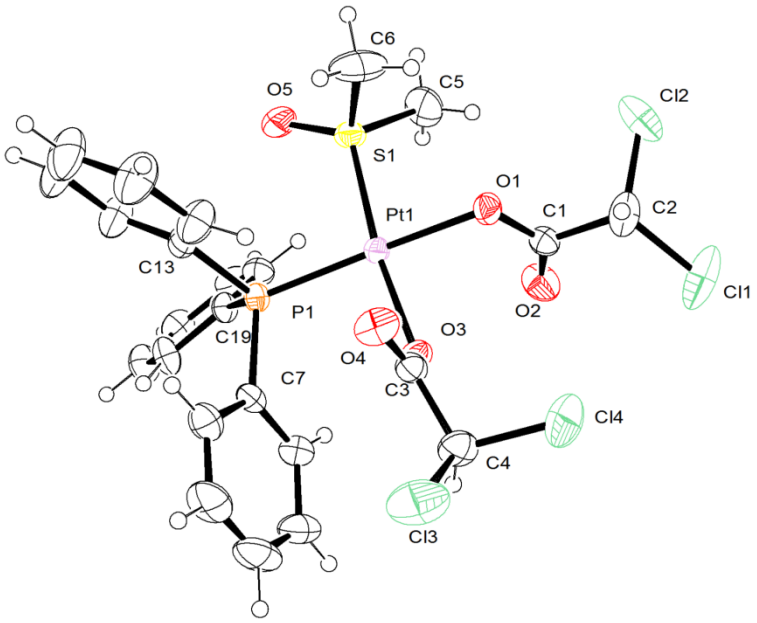


Figure 2

ORTEPIII [10] view and atom numbering scheme for complex **3,** *cis*-[Pt(DCA)$_2$(PPh$_3$)(Me$_2$SO-*S*)]. Thermal ellipsoids are drawn at the 50% probability level.

The same reaction with two equivalents of PPh$_3$ gave complex *cis*-[Pt(DCA)$_2$(PPh$_3$)$_2$], **3a,** whose ^{31}P NMR in acetone showed a singlet with satellites at 5.13 ppm with $^1J_{PPt}$ = 3873 Hz, typical of a Pt-coordinated PPh$_3$ *trans* to a carboxylate [11], which proves the *cis* configuration for **3a**. In ^{1}H NMR, the signal of coordinated DCA was found at 5.35 ppm.

Finally, the addition of three equivalents of PPh_3 to *cis*-[Pt(DCA)$_2$(Me_2SO-*S*)$_2$] gave the cationic complex [Pt(DCA)(PPh_3)$_3$]DCA, **3b**, as unequivocally indicated by its ^{31}P NMR in acetone, showing a triplet at 2.77 ppm and a doublet at 22.59 ppm reciprocally coupled ($^2J_{PAPB}$ 19.3 Hz) and coupled to ^{195}Pt ($^1J_{PtP}$ 3626 Hz and 2583 Hz respectively). The ^1H NMR presents two signals for DCA, Pt-coordinated at 3.81 ppm and uncoordinated $CHCl_2COO^-$ at 6.0 ppm, the same value found for $CHCl_2COONa$ (6.0 ppm in D_2O).

Complex **3a** has been characterized by X-ray crystallographic analysis (Fig. 3 and Table 1)

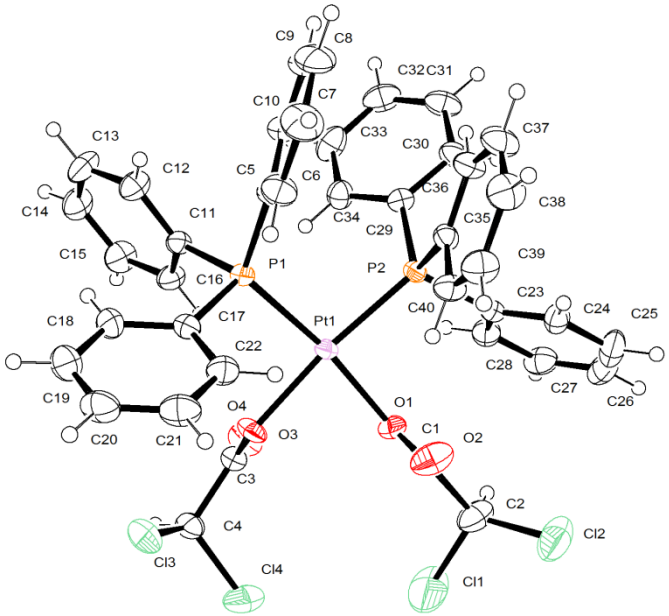


Figure 3

ORTEPIII [10] view and atom numbering scheme for complex **3a,** *cis*-[Pt(DCA)$_2$(PPh_3)$_2$]. Thermal ellipsoids are drawn at the 50% probability level. The solvent molecule is not shown for the sake of clarity.

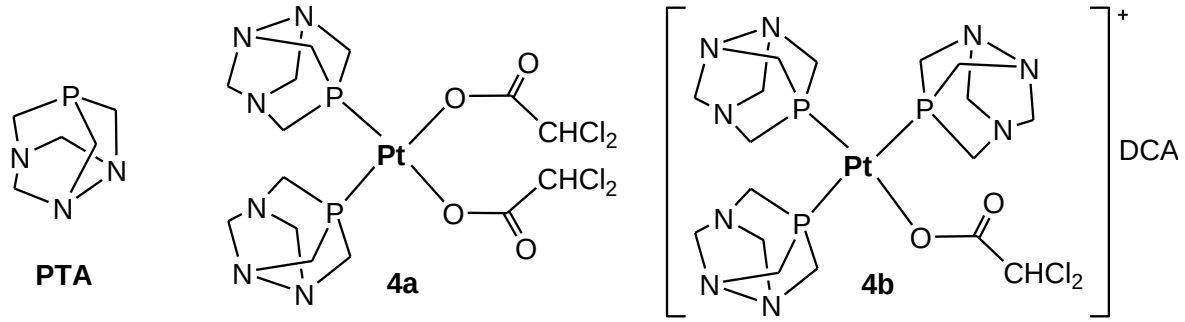
When we tried the substitution of Pt-bonded DMSO with PPh_3, we were aware of possible problems due to the fact that the substitution of the ligand in trans to DMSO is often favored over the substitution of DMSO itself. Indeed when we tried to replace DMSO in complex **1** [$PtCO_3(DMSO)_2$], we got mixture of products where probably water is involved too.

On the contrary, when we replaced CO_3^{2-} with RCOOH, the following substitution of DMSO in cis-[Pt(RCOO)$_2$($DMSO$)$_2$] occurred smoothly.

The DMSO trans effect has been reported since the early seventies, but the possibility to replace DMSO in a square planar Pt complex has also been documented [12].

*2.3. PTA-DCA-Pt complexes. X-ray crystal structure of cis-[Pt(DCA)$_2$(PTA)$_2$] (**4a**)*

PTA (1,3,5-triaza-7-phosphaadamantane) is an aliphatic phosphine which has attracted a great interest mainly because of its ability of increasing the water solubility of complexes with respect to aromatic phosphines analogues, maintaining their stability toward oxidation [13].



As described above for **3a**, also complex *cis*-[Pt(DCA)$_2$(PTA)$_2$], **4a,** can be prepared in "one pot", in acetone under nitrogen, from complex **1** plus two equivalents of DCA giving the above described complex **2** as a non-isolated intermediate; this yielded **4a** by addition of two equivalents of PTA. The addition of 1 eq of PTA gave a mixture of *cis*-[Pt(DCA)$_2$(Me$_2$SO-*S*)$_2$] (**2)** and *cis*-[Pt(DCA)$_2$(PTA)$_2$] (**4a**)**.** The expected mixed complex *cis*-[Pt(DCA)$_2$(Me$_2$SO-*S*)(PTA)] was not obtained in a variety of conditions.

The new complex **4a** is of great interest because it gathers on platinum two advantageous ligands: DCA, which it is known to trigger apoptosis in cancer cells [2], and PTA, regarded as a rare example of biocompatible hydrophilic phosphine [14].

Complex **4a** was characterized by NMR. In the ^{1}H NMR in DMSO the signals of coordinated PTA (one singlet at 4.25 and two doublets at 4.41 and 4.43 ppm due to unequivalent NCH$_2$N) and the signal of CHCl$_2$ as a broad peak at 6.25 ppm were observed. The ^{13}C NMR in DMSO showed the signals of PTA at 50.0 (NCH$_2$P, $^1J_{CP}$ = 24.4 Hz) and 71.59 (NCH$_2$N) ppm, together with the CHCl$_2$ signal at 69.15 ppm and of COO at 165.77 (C-Pt coupling not visible due to low signal to noise ratio) ppm.

The ^{31}P NMR in DMSO showed a singlet with satellites at -60.60 ppm with a $^{1}J_{PPt}$ = 3417 Hz, supporting a *cis* configuration for **4a**.

In the MS-ESI spectrum of **4a**, the main peak was found at m/z = 636.93, corresponding to the monopositive cation formed by loss of one DCA (MW – $CHCl_2COO + H^+$).

Crystals of **4a** suitable for x-ray analysis grew in DMSO (Fig 4 and Table 1).

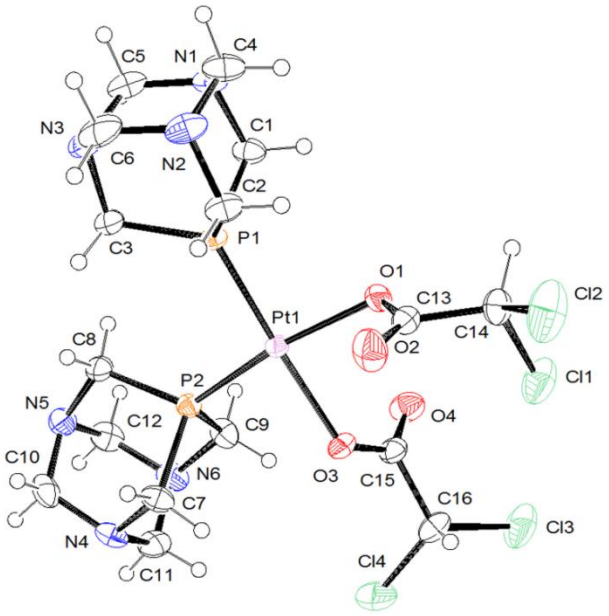


Figure 4

ORTEPIII view [10] and atom numbering scheme for *cis*-[Pt(DCA)$_2$(PTA)$_2$], **4a**. Thermal ellipsoids are drawn at the 50% probability level. The solvent molecule is not shown for the sake of clarity.

The addition of three equivalents of PTA to *cis*-[Pt(DCA)$_2$(Me$_2$SO-*S*)$_2$], **2**, gave the cationic complex [Pt(DCA)(PTA)$_3$]DCA, **4b**, as indicated by its ^{31}P NMR (a triplet and a doublet reciprocally coupled and coupled to ^{195}Pt: -59.48 (t, $^{1}J_{PtP}$ = 3225 Hz) ppm, δ = -52.88 (d, $^{1}J_{PtP}$ = 2224 Hz) ppm, $^{2}J_{PAPB}$ = 22.5 Hz. The cationic nature of **4b** is a favorable factor for an efficient approach to anionic DNA and consequent alkylating effect.

2.4 Crystal structure description

ORTEPIII [10] views of the structure of **2**, **3, 3a** and **4a** are shown in Figures 1, 2, 3 and 4, respectively, while selected bond lengths and angles are reported in Table 1. **2**, **3**, **3a** and **4a** are the first examples of structurally characterised non amine Pt complexes of DCA, and only three crystal structures of Pt-DCA-amine complexes have been reported so far [3b, 3c].

In **2** the asymmetric unit is formed by two Pt complexes, while in **3a** and **4a** a cocrystallized solvent molecule (acetone and DMSO, respectively) is also present.

All complexes present a slightly distorted square-planar geometry, with the metal centre bound to two *cis* dichloroacetate molecules (acting as monodentate ligands), two sulfur (**2**), one sulfur and one phosphorous (**3**) and two phosphorus (**3a** and **4a**) atoms. The greater distortion (Table 1) is found in complexes **3a** and **4a**, due to the steric hindrance of the triphenylphosphine or PTA ligands in *cis* position (Figs 3 and 4). In all complexes the Pt-O distances are quite similar, varying in the narrow range 2.040(3) - 2.089(2) Å, and are in good agreement with the mean value of 2.02(2) Å calculated for Pt-acetate complexes (115 hits in CSD). The second oxygen of the carboxylate group is in all cases more than 3.2 Å far away from the metal.

Due to the lack of good hydrogen bonding donors, the crystal packing of the four complexes is characterized by the presence of a number of weak C-H…O interactions, listed in Table 2. The only exception is the Cl…Cl interaction found in **2**. Halogen···halogen R1-X…X-R2 contacts are characterized by an interhalogen distance that is less than the sum of the van der Waals radii. It has been shown that there are two preferred geometries for such interactions [15]: the first occurs when $\theta_1=\theta_2$, (where θ_1 and θ_2 are the R1-X…X and X…X-R2 angles, respectively) while the second occurs when $\theta_1\approx180°$ and $\theta_2\approx90°$. In complex **2** the second 'perpendicular' arrangement has been found, being the C10-Cl5…Cl1 and Cl5…Cl1-C2 angles of 172 and 105 °, respectively (Fig. 5)

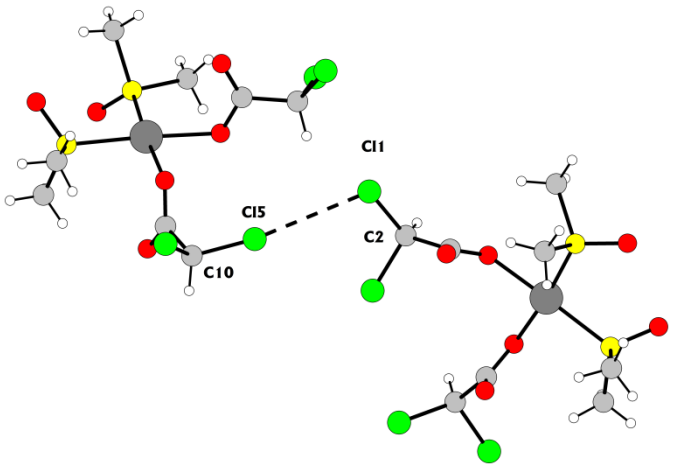


Figure 5. Cl…Cl halogen bond in complex **2**

Table 1. Selected bond distances and angles (Å, °) for complexes **2, 3, 3a** and **4a**

Complex 2

Pt1 - S1	2.226(1)	Pt2 - S3	2.221(1)
Pt1 - S2	2.223(1)	Pt2 - S4	2.217(1)
Pt1 - O1	2.044(3)	Pt2 - O7	2.046(4)
Pt1 - O4	2.047(4)	Pt2 - O9	2.043(3)
S1 - Pt1 - S2	92.19(4)	S3 - Pt2 - S4	91.09(4)
S1 - Pt1 - O1	171.8(1)	S3 - Pt2 - O7	93.2(1)
S1 - Pt1 - O4	93.0(1)	S3 - Pt2 - O9	175.8(1)
S2 - Pt1 - O1	94.1(1)	S4 - Pt2 - O7	175.5(1)
S2 - Pt1 - O4	174.7(1)	S4 - Pt2 - O9	93.1(1)
O1- Pt1 - O4	80.7(1)	O7 - Pt2 - O9	82.6(1)

Complex 3

Pt1 - P1	2.251(1)	Pt1 - O3	2.040(3)
Pt1 - S1	2.197(1)	P1 - C13	1.819(4)
Pt1 - O1	2.072(3)	P1 - C19	1.810(4)
P1 - Pt1 - S1	95.37(4)	S1 - Pt1 - O1	88.42(8)
P1 - Pt1 - O1	172.41(8)	S1 - Pt1 - O3	172.51(8)
P1 - Pt1 - O3	92.06(8)	O1 - Pt1 - O3	84.09(9)

Complex 3a

Pt1 - O1	2.081(2)	P1 - C11	1.819(3)
Pt1 - O3	2.076(3)	P1 - C17	1.819(3)
Pt1 - P1	2.256(1)	P2 - C23	1.828(3)
Pt1 - P2	2.236(1)	P2 - C29	1.827(3)
P1 - C5	1.829(3)	P2 - C35	1.814(3)
O1 - Pt1 - O3	84.1(1)	O3 - Pt1 - P1	87.99(8)
O1 - Pt1 - P1	171.78(8)	O3 - Pt1 - P2	173.59(7)
O1 - Pt1 - P2	90.35(7)	P1 - Pt1 - P2	97.40(3)

Complex 4a

Pt1 - P1	2.217(1)	P1 - C2	1.838(4)
Pt1 - P2	2.212(1)	P1 - C3	1.841(4)
Pt1 - O1	2.087(3)	P2 - C7	1.838(4)
Pt1 - O3	2.089(2)	P2 - C8	1.832(4)
P1 - C1	1.838(4)	P2 - C9	1.837(4)
P1 - Pt1 - P2	100.12(4)	P2 - Pt1 - O1	169.11(7)
P1 - Pt1 - O1	89.10(7)	P2 - Pt1 - O3	86.67(8)
P1 - Pt1 - O3	170.55(7)	O1 - Pt1 - O3	84.87(10)

Table 2. Hydrogen bonding parameters (Å, °) for complexes **2, 3, 3a** and **4a**

	D-H	D....A	H....A	D-H...A
Complex 2				
C5-H...O3	0.96	3.393(6)	2.63	136
C7-H...O2	0.96	3.136(9)	2.34	140
C13-H...O8	0.96	3.348(7)	2.54	141
C4-H...O5^{i}	0.98	3.283(7)	2.35	157
C5-H...O12^{i}	0.96	3.432(7)	2.60	145
C6-H...O12^{i}	0.96	3.281(7)	2.36	159
C10-H...O10^{ii}	0.98	3.242(7)	2.26	173
C16-H...O9^{iii}	0.96	3.367(6)	2.41	173
C13-H...O6^{iv}	0.96	3.499(7)	2.58	158
C15-H...O8^{v}	0.96	3.384(7)	2.51	151
Short Cl…Cl contact:				
Cl1 ...Cl5^{iii}		3.396(2)		

Equivalent positions: (i) 2 -x,1-y,1-z; (ii) x-1,y,z; (iii) 1-x,-y,-z; (iv) 1-x,1-y,1-z; (v) x+1,y,z

Complex 3

C14-H...O4	0.93	3.528(6)	2.63	160
C6-H...O2^{i}	0.96	3.192(6)	2.46	132

Equivalent positions: (i) x-1/2,y,3/2-z

Complex 3a

C16-H...O4	0.93	3.537(5)	2.65	159
C40-H...O2	0.93	3.346(4)	2.47	156
C32-H...O4^{i}	0.93	3.303(5)	2.59	134

Equivalent positions: (i) 1-x,1-y,-z

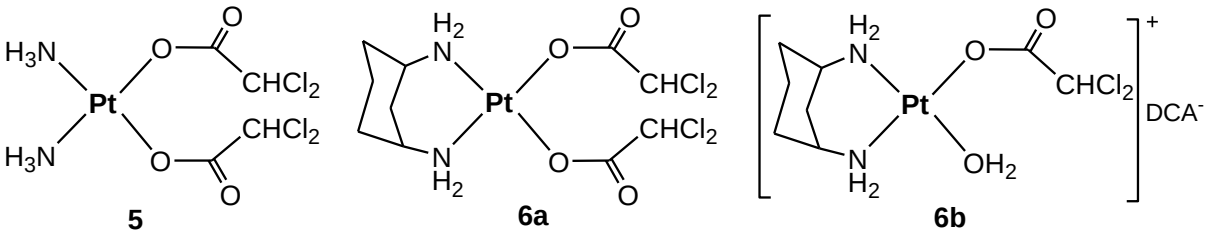
Complex 4a

C2 -H ...O5	0.97	3.427(7)	2.50	159
C8-H ...O4^{i}	0.97	3.432(7)	2.58	146
C3-H ...O2^{ii}	0.97	3.227(7)	2.38	144

Equivalent positions: (i) 1-x,-y,1-z; (ii) -x,-y,1-z

2.4 Synthesis and characterization of aminic Pt-DCA complexes.

For comparative tests, the known amine complexes *cis*-[Pt(DCA)$_2$(NH$_3$)$_2$], **5,** [Pt(DCA)$_2$(1,2-DACH)], **6a,** and [Pt(DCA)(H$_2$O)(1,2-DACH)$_2$]DCA, **6b,** have been prepared by a modified version of the reported procedure [4, 15].



2.5 Antiproliferative activity tests

The antiproliferative activity of complexes **2**, **4a**, **4b, 5, 6a and 6b** were tested on two human cancer cell lines: A2780 and SKOV. 50 mM stock solution of each complex were prepared in DMSO and stored at -18°C; the working solutions (5 mM, 500 μM and 50 μM) were obtained using EtOH.

Although the use of DMSO is controversial because of its propensity to react with Pt(II) compounds modifying the structure of the original compound, the observed invariance of the NMR spectra (after 3 days) in the presence of DMSO excludes this possibility. The aminic complexes *cis*-[Pt(DCA)$_2$(NH$_3$)$_2$], **5,** [Pt(DCA)$_2$(1,2-DACH)], **6a,** and [Pt(DCA)(H$_2$O)(1,2-DACH)$_2$]DCA, **6b,** have been tested as comparison. Untreated cells (also treated with DMSO) were used as a negative control.

On Pt sensitive A2780, the non-amine complexes **2, 4a** and **4b** are slightly less active then the amine complexes **5**, **6a** and **6b**. Nevertheless, moving to Pt resistant SKOV-3, the amine complexes **5, 6a** and **6b** were found appreciably less active (resembling cisplatin behavior), while **2, 4a** and **4b** showed the same activity. This could indicate that the amine complexes **5**, **6a** and **6b** act through a mechanism similar to that of cisplatin, while **2, 4a** and **4b** express a different action.

The comparison with the series of dichloride analogues, that is *cis*-[PtCl$_2$(Me$_2$SO-*S*)$_2$], *cis*-[PtCl$_2$(PTA)$_2$], [PtCl$_2$(1,2-DACH)] and cisplatin, is also reported in Table 3 for both cell lines. It shows that, in the group of the amine complexes (**5**, **6a** and **6b)** the introduction of DCA has no effect on the antiproliferative activity against Pt sensitive A2780, but increases the antiproliferative activity on Pt-resistant SKOV-3, with respect with the corresponding dichloride; on the other hand, in the case of **2, 4a** and **4b,** the introduction of DCA seems to generate an appreciable

antiproliferative activity, on both cell lines, with respect to the inactive parent dichlorides *cis*-[$PtCl_2(Me_2SO\text{-}S)_2$] and *cis*-[$PtCl_2(PTA)_2$].

Table 3

Estimated IC_{50} (μM) on A2780 and SKOV3 cell lines

complex	**IC_{50} (μM) A2780**	**IC_{50} (μM) SKOV3**
2	2.7±0.65	3±0.46
cis-[$PtCl_2(Me_2SO\text{-}S)_2$]	> 50	> 50
4a	5.3±0.76	5.4±0.55
4b	3.7±0.60	4.0±0.89
cis-[$PtCl_2(PTA)_2$],	> 50	> 50
5	1.9±0.08	4.7±1.21
6a	1.3±0.37	4.5±1.06
6b	1.8±1.15	5.7±1.36
[$PtCl_2$(1,2-DACH)]	0.4±0.02	8.9±0.08
cis-[$PtCl_2(NH_3)_2$]	2.9±0.18	5.9±0.06

3. Experimental

3.1. General

All the manipulations were carried out in the air atmosphere unless otherwise noted. Commercial solvents and reagents were purchased and used without further purification. PTA [17], the precursors *cis*-[$PtCl_2(Me_2SO\text{-}S)_2$] [18], *cis*-[$PtCO_3(Me_2SO\text{-}S)_2$] [7], *cis*-[$PtCl_2(PTA)_2$] [19], [$PtCl_2$(1,2-DACH)] [20] and *cis*-[$PtCl_2(NH_3)_2$] [21] were prepared as described in the literature. The amine-DCA complexes **5**, **6a** and **6b** were prepared by a modified version (below described) of the reported procedure [4, 16].

Elemental analyses were determined using a Carlo Erba instrument model EA1110. The ESI mass spectra were acquired with a Micromass LCQ Duo Finningan. NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer (^{1}H at 300 MHz, ^{13}C at 75.43 MHz, ^{31}P at 121.50 MHz) or a Varian Mercury Plus (^{1}H at 400 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.92 MHz, ^{195}Pt at 85.64 MHz). The ^{13}C, ^{31}P and ^{195}Pt spectra were run with proton decoupling; ^{13}C signals are reported in ppm relative to external tetramethylsilane (TMS), ^{31}P signals are reported in ppm relative to an external 85% H_3PO_4 standard and the reference for ^{195}Pt NMR was Na_2PtCl_6 1M in D_2O.

3.2. Synthesis of complex **2,** *cis*-[$Pt(DCA)_2(Me_2SO\text{-}S)_2$]

Dichloroacetic acid (0.040 mL, $4.86 \cdot 10^{-4}$ mol, MW 128.9 g/mol, d = 1.56 g/ml, 2 eq) was added to a solution of complex [$PtCO_3(Me_2SO\text{-}S)_2$], **1**, (100 mg, $2.43 \cdot 10^{-4}$ mol, MW 411.2 g/mol) in 10 mL of CH_3OH, under vigorous stirring. A white solid precipitated in 20 minutes. The suspension was kept under stirring for further 2 hours and the white solid was separated by filtration and dried under vacuum over P_2O_5 (0.14 g, $2.30 \cdot 10^{-4}$ mol, MW 607.2 g/mol, yield 95%). Anal. Calc. (%) for $C_8H_{14}Cl_4O_6PtS_2$ (607.2): C, 15.82; H, 2.32; S, 10.56%. Found: C,15.93; H, 2.45; S, 10.23 %. ^{1}H NMR (acetone-d_6): 3.53 ppm (s, $^3J_{HPt}$ = 22 Hz, 12H, DMSO), 6.15 ppm (s, 2H, $CHCl_2$). ^{13}C NMR (acetone-d_6): 43.63 ppm (CH_3, DMSO), 68.01 ppm (s, $CHCl_2$), 169.08 ppm (s, COO). ^{195}Pt NMR: δ = -3119 ppm.
The crystallographic structure was determined on crystals of **2** grown in acetone (Fig. 1 and Table 1).

3.3. Synthesis of PPh_3-DCA complexes [$Pt(DCA)_2(PPh_3)(Me_2SO\text{-}S)$]**, 3**, [$Pt(DCA)_2(PPh_3)_2$], **3a,** *and* **[**$Pt(DCA)(PPh_3)_3$]DCA**, 3b.**

The carbonate complex **1** [$PtCO_3(Me_2SO\text{-}S)_2$] (100 mg, $2.43 \cdot 10^{-4}$ mol, MW 411.2 g/mol) was solubilized in 40 mL of acetone and pure dichloroacetic acid (0.040 mL, $4.86 \cdot 10^{-4}$ mol, MW 128.9 g/mol, 2 eq) was added. After 1 hour stirring, PPh_3 (64 mg, $2.43 \cdot 10^{-4}$ mol, MW 262.3 g/mol, 1 eq) was solubilized in 5 mL of acetone and added to the previous solution.
After 2 hours of further stirring, the solution was taken to dryness and complex **3** was obtained as a white oil, that crystallized from CH_2Cl_2 and Et_2O. (0.120 g, $1.50 \cdot 10^{-4}$ mol, MW 791.4 g/mol, yield 62 %). Anal. Calc. (%) for $C_{24}H_{23}Cl_4O_5PPtS$ (791.4): C, 36.42; H, 2.93; S, 4.05%. Found: C, 36.33; H, 2.95; S, 4.13 %. ^{1}H NMR (acetone-d_6): 3.22 ppm (s, 63H, DMSO, $^3J_{HPt}$ = 21 Hz), 5.38 ppm (s, 1H, $CHCl_2$ trans to P), 6.18 ppm (s, 1H, $CHCl_2$ trans to S), 7.50-7.90 ppm (m, 15H, PPh_3). ^{1}H NMR ($CDCl_3$): 3.10 ppm (bs, 6H, DMSO), 5.28 ppm (s, 1H, $CHCl_2$ trans to P), 5.90 ppm (s, 1H, $CHCl_2$ trans to S), 7.40 - 7.90 ppm (m, 15H, PPh_3). ^{31}P NMR (acetone -d_6): 7.9 ppm (s, $^1J_{PtP}$ = 4015 Hz, PPh_3). ^{31}P NMR ($CDCl_3$): 7.1 ppm (s, $^1J_{PtP}$ = 3956 Hz, PPh_3). ^{31}P NMR (DMSO-d_6): 8.0 ppm (s, $^1J_{PtP}$ =4030 Hz, PPh_3).
The crystallographic structure was determined on crystals of **3** grown in DMSO (Fig.2 and Table 1).

In a similar experiment, two equivalents of PPh_3 (127 mg, $4.86 \cdot 10^{-4}$ mol) were used, keeping all the other quantities and conditions the same as above. Complex **3a** was obtained (0.185 g, $1.9 \cdot 10^{-4}$ mol, MW 975.5 g/mol, yield 78.2 %) and characterized by ^{1}H and ^{31}P NMR.
Anal. Calc. (%) for $C_{40}H_{32}Cl_4O_4P_2Pt$ (975.5): C, 49.25; H, 3.31 %. Found: C, 49.56; H, 3.30 %. ^{1}H NMR (acetone-d_6): 5.35 ppm (s, 2H, $CHCl_2$ trans to P), 7.20-7.90 ppm (m, 30H, PPh_3). ^{1}H NMR ($CDCl_3$): 5.28 ppm (s, 2H, $CHCl_2$ trans to P), 7.00-7.60 ppm (m, 30H, PPh_3).^{31}P NMR (acetone -d_6): 5.13 ppm (s, $^{1}J_{PtP} = 3873$Hz, PPh_3). ^{31}P NMR ($CDCl_3$): 5.16 ppm (s, $^{1}J_{PtP} = 3882$ Hz, PPh_3). ^{31}P NMR (DMSO-d_6): 5.17 ppm (s, $^{1}J_{PtP} = 3878$ Hz).
The crystallographic structure was determined on crystals of **3a** grown in acetone (Fig.3 and Table 1).

In a parallel experiment, three equivalents of PPh_3 (192 mg, $7.32 \cdot 10^{-4}$ mol) were used, leaving all the other quantities and conditions the same as above. Complex **3b** was obtained (0.272 g, $2.2 \cdot 10^{-4}$ mol, MW 1237.8 g/mol, yield 90.5 %). Anal. Calc. (%) for $C_{58}H_{47}Cl_4O_4P_3Pt$ (1237.8): C, 56.28; H, 3.83 %. Found: C, 55.98; H, 4.01 %. ^{1}H NMR (acetone-d_6): 3.81 ppm (s, 1H, $CHCl_2$ trans to P), 6.0 ppm (s, 1H, $CHCl_2COO^-$), 7.00-7.60 ppm (m, 30H, PPh_3). ^{1}H NMR ($CDCl_3$): 3.85 ppm (s, 1H, $CHCl_2$ trans to P), 6.1 ppm (s, 1H, $CHCl_2COO^-$), 7.00-7.60 ppm (m, 30H, PPh_3). ^{31}P NMR (acetone-d_6): 2.77 ppm (t, $^{1}J_{PtP} = 3626$ Hz, P_APh_3), 22.59 ppm (d, $^{1}J_{PtP} = 2583$ Hz, P_BPh_3), $^{2}J_{PAPB} = 19.3$ Hz.^{31}P NMR ($CDCl_3$): 2.79 ppm (t, $^{1}J_{PtP} = 3629$ Hz, P_APh_3), 22.50 ppm (d, $^{1}J_{PtP} = 2585$ Hz, P_BPh_3), $^{2}J_{PAPB}$ 19.3 Hz. ^{31}P NMR (DMSO-d_6): 2.62 ppm (t, $^{1}J_{PtP} = 3590$ Hz, P_APh_3), 22.17 (d, $^{1}J_{PtP} = 2600$ Hz, P_BPh_3), $^{2}J_{PAPB}$ 19.1 Hz .

3.4. Synthesis of PTA-DCA complexes cis-$[Pt(DCA)_2(PTA)_2]$, **4a,** *and cis*-$[Pt(DCA)(PTA)_3]DCA$, **4b.**

The Pt-carbonate complex **1**, $[PtCO_3(Me_2SO\text{-}S)_2]$, (62 mg, $1.51 \cdot 10^{-4}$ mol, MW 411.2 g/mol) was suspended in 40 mL of acetone. Pure dichloroacetic acid (0.025 mL, $3.01 \cdot 10^{-4}$ mol, MW 128.9 g/mol, 2 eq) was added to the suspension, which turned into a clear solution containing the intermediate *cis*-$[Pt(DCA)_2(Me_2SO\text{-}S)_2]$, **2** ($1.51 \cdot 10^{-4}$ mol, assuming a total conversion). PTA (47 mg, $3.0 \cdot 10^{-4}$ mol, MW 157.1 g/mol, 2 eq), dissolved in 15 mL of acetone, was added under nitrogen. A white solid immediately started to precipitate and the mixture was kept under vigorous stirring for two hours. Complex **4a**, a white solid, was filtered and dried under vacuum (90 mg, $1.18 \cdot 10^{-4}$ mol, MW 765.2 g/mol, yield 78%). Anal. Calc. (%) for

$C_{16}H_{26}Cl_4N_6O_4P_2Pt$ (765.2): C, 25.11; H, 3.42; N, 10.98%. Found: C, 25.52; H, 3.42; N, 11.28%.^{1}H NMR (DMSO-d_6): 4.25 ppm (s, 12 H, PTA), 4.41 and 4.42 ppm (2d, 12H, PTA), 6.25 ppm (s, 2H, DCA). ^{13}C NMR (DMSO-d_6): 50.00 ppm (d, $^1J_{CP}$ = 24.4 Hz, NCH_2P), 69.15 ppm (s, $CHCl_2$), 71.59 ppm (s, NCH_2N), 165.77 ppm (s, COO). ^{31}P NMR (DMSO): -60.60 ppm (bs, $^1J_{PtP}$ = 3417 Hz).^{195}Pt (DMSO): -3383 ppm (t, $^1J_{PtP}$ = 3418 Hz) ppm. MS-ESI: m/z = 636.93 (MW-$CHCl_2COO^-$ + H^+).

Crystals suitable for x-ray analysis grew in DMSO in two weeks and the crystallographic structure of **4a** has been determined (Fig.4 and Table 1).

In an analogue experiment, 3 eq of PTA (71 mg, $4.53 \cdot 10^{-4}$ mol, MW 157.1 g/mol) were added to a solution containing **2** ($1.51 \cdot 10^{-4}$ mol), obtained as above for **4a**. After the same work up, **4b** was obtained as a white solid. (116 mg, $1.25 \cdot 10^{-4}$ mol, MW 922.4 g/mol, yield 83%). Anal. Calc. (%) for $C_{22}H_{38}Cl_4N_9O_4P_3Pt$ (922.4): C, 28.65; H, 4.15; N, 13.67%. Found: C, 28.83; H, 4.12; N, 13.87%. ^{1}H NMR (DMSO-d_6): 4.25 and 4.42 ppm (2m, 36H, PTA), 6.05 ppm (s, 2H, DCA). ^{31}P NMR (DMSO) -59.48 ppm (t, $^1J_{PtP}$ = 3225 Hz), -52.88 ppm (d, $^1J_{PtP}$ = 2224 Hz), $^2J_{PAPB}$ = 22.5 Hz.

3.5. Synthesis of amine DCA complexes **5**, **6a** and **6b**

Complex cis-[$Pt(DCA)_2(NH_3)_2$], **5**

Cis-[$PtI_2(NH_3)_2$] (0.2 g, $4.13 \cdot 10^{-4}$ mol, MW 483.1 g/mol) was suspended in 70 mL of water and solid Ag_2CO_3 (0.114 g, $4.13 \cdot 10^{-4}$ mol, 276 g/mol) was added at 50°C. After one hour stirring, AgI was eliminated by filtration and DCA (0.106 g, $8.26 \cdot 10^{-4}$ mol, MW 128.9 g/mol, 0.068 mL) was added to the solution. It was left at r.t. under stirring overnight and then taken to dryness, leaving **5** as a white solid (0.18 g, $3.71 \cdot 10^{-4}$ mol, MW 485.0 g/mol, yield 90%). ^{1}H NMR in acetone-d_6: 4.68 ppm (bs, NH_3, 6H), 6.03 ppm (s, CH, 2H).

Complex [$Pt(DCA)_2$(1,2-DACH)], **6a**

A solution of complex [$PtCO_3$(1,2-DACH)], (80 mg, $2.16 \cdot 10^{-4}$ mol, MW 369.1 g/mol) in 10 mL of H_2O was prepared and dichloroacetic acid (0.036 mL, $4.32 \cdot 10^{-4}$ mol, MW 128.9 g/mol, d = 1.56 g/ml, 2 eq) was added under vigorous stirring. Complex **6a** formed in 1 h as a white solid which was separated by centrifugation, washed with water and dried under vacuum over P_2O_5 (**6a**, 0.12 g, $2.12 \cdot 10^{-4}$ mol, MW 565.1 g/mol, yield 98%). Anal. Calc. (%)

for $C_{10}H_{16}Cl_4N_2O_4Pt$ (565.1): C, 21.25; H, 2.85; N, 4.96%. Found: C, 21.35; H, 2.95; N, 4.98%.

^{1}H NMR (DMSO-d_6): 1.0 ppm (m, 2H), 1.3 ppm (m, 2H), 1.45 ppm (m, 2H), 1.9 ppm (m, 2H), 2.32 ppm (m, 2H), 5.82 ppm (s, 2H, $CHCl_2$).

Complex [Pt(DCA)(H_2O)(1,2-DACH)$_2$]DCA, **6b**

Complex [$PtCO_3$(1,2-DACH)], (110 mg, $2.9 \cdot 10^{-4}$ mol, MW 369.1 g/mol) was dissolved in 25 mL of H_2O and dichloroacetic acid (0.024 mL, $2.9 \cdot 10^{-4}$ mol, MW 128.9 g/mol, d = 1.56 g/mL, 1 eq) was added. After 4 h, a second equivalent of dichloroacetic acid (0.024 mL as above, 1 eq) was added. The solution was kept under stirring overnight and then taken to dryness, leaving an oily residue of **6b,** which was dried under vacuum over P_2O_5 (0.12 g, $2.64 \cdot 10^{-4}$ mol, MW 454 g/mol, yield 91%). Anal. Calc. (%) for $C_{10}H_{18}Cl_4N_2O_5Pt$ (583.1): C, 20.60; H, 3.11; N, 4.80%. Found: C, 21.05; H, 2.97; N, 4.87%.^{1}H NMR (DMSO-d_6): 1.0 ppm (m, 2H), 1.3 ppm (m, 2H), 1.45 ppm (m, 2H), 1.9 ppm (m, 2H), 2.32 ppm (m, 2H), 5.82 ppm (s, 1H, $CHCl_2$), 6.5 ppm (s, 1H, $CHCl_2$). MS-ESI: m/z = 514.86 (MW-H_2O + DMSO), 436.96 (MW-H_2O).

3.6 X-Ray Crystallography

Single-crystal diffraction data for complexes **2**, **3**, **3a** and **4a** were collected on a Nonius Kappa diffractometer equipped with a CCD detector with graphite-monochromatized MoKα radiation (λ = 0.71069 Å). Intensities were corrected for Lorentz, polarization and absorption effects.[22] The structures were solved by direct methods with the SIR97 suite of programs [23] and refinement were performed on F^2 by full-matrix least-squares methods with all non-hydrogen atoms anisotropic. In all stuctures the hydrogen atoms were included on calculated positions, riding on their carrier atoms. All calculations were performed using SHELXL-97 [24], implemented in the system of programs WINGX [25]. Crystal data are reported in Table 4.

Table 4. Experimental details

Complex	**2**	**3**	**3a**	**4a**
Chemical formula	$C_8H_{14}Cl_4O_6PtS_2$	$C_{24}H_{23}Cl_4O_5PPtS$	$C_{41}H_{31}Cl_4O_4P_2Pt \cdot C_2H_6O$	$C_{16}H_{26}Cl_4N_6O_4P_2Pt \cdot C_2H_6OS$
M_r	607.20	791.34	1032.55	843.38
Crystal system, space group	Triclinic, *P-1*	Orthorhombic, *Pbca*	Triclinic, $P\bar{1}$	Monoclinic, $P2_1/c$
Temperature (K)	295	295	295	295
a, *b*, *c* (Å)	8.4710 (1), 11.2254 (1), 19.3097 (2)	13.9642 (3), 17.2510 (4), 23.5520 (5)	12.1612 (2), 13.3503 (2), 13.9202 (2)	10.7809 (2), 11.6091 (3), 23.4627 (5)
α, β, γ (°)	106.854 (1), 92.546 (1), 91.486 (1)	90, 90, 90	76.4880 (8), 70.7940 (7), 84.8830 (9)	90, 102.962 (1), 90
V (Å^3)	1754.11 (3)	5673.6 (2)	2074.98 (6)	2861.7 (1)
Z	4	8	2	4
μ (mm^{-1})	8.86	5.49	3.76	5.50
Crystal size (mm)	0.24 × 0.15 × 0.06	0.52 × 0.29 × 0.14	0.50 × 0.35 × 0.12	0.23 × 0.12 × 0.06
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	39355, 8452, 7680	28966, 6831, 5301	45656, 9947, 9189	27162, 6897, 5717
R_{int}	0.086	0.079	0.059	0.059
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.035, 0.100, 1.04	0.034, 0.089, 1.06	0.031, 0.082, 1.04	0.030, 0.069, 1.04
No. of reflections	8452	6831	9947	6897
No. of parameters	380	325	496	334
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	2.52, -2.18	1.13, -1.63	1.51, -2.36	1.42, -1.43

3.7 Growth inhibition assays

Cell growth inhibition assays were carried out using two human ovarian cancer cell lines, A2780 and SKOV3; A2780 cells are cisplatin-sensitive and SKOV3 cells are cisplatin-resistant. Cell lines were obtained from ATCC (Manassas, VA) and maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL^{-1}), streptomycin (100 μg mL^{-1}) and glutamine (2 mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37°C in a 5% CO_2 atmosphere. Adherent cells were routinely used at 70% of confluence and passaged every 3 days by treatment with 0.05% trypsin-EDTA (Lonza).

Pure derivatives were added at serial dilutions and incubated for 3 days. After this time, cells were washed with PBS 1X and detached with trypsin. Cells were suspended in physiological solution and counted with a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/ml was determined as IC_{50} after 3 days of culture, when untreated cells are in log phase of cell growth [26]. Stock solutions (50 mM) of compounds were made in DMSO, while working solutions (5 mM, 500 μM and 50 μM) were obtained using EtOH. All solutions were diluted in complete medium to give final concentrations. Cisplatin was employed as a control for the cisplatin-sensitive A2780, and for the cisplatin-resistant SKOV3. Untreated cells were placed in every plate as negative control. The cells were exposed to the compounds in 1000 μL total volume, for 72 hours.

4. Conclusion

A group of new Pt complexes containing the proapoptotic anion dichloroacetate have been prepared and characterized. **2**, **3**, **3a** and **4a** are the first examples of structurally characterised Pt complexes of DCA without N donors. It has been found that their antiproliferative activity is compable with that of cisplatin. This results encourage a further exploration of the anticancer activity of these compounds.

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Supplementary material

CCDC **1531776-1531779** contains the supplementary crystallographic data for complexes **2, 3, 3a** and **4a**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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